

Recent Developments of Carbon Dots in Biosensing: A Review

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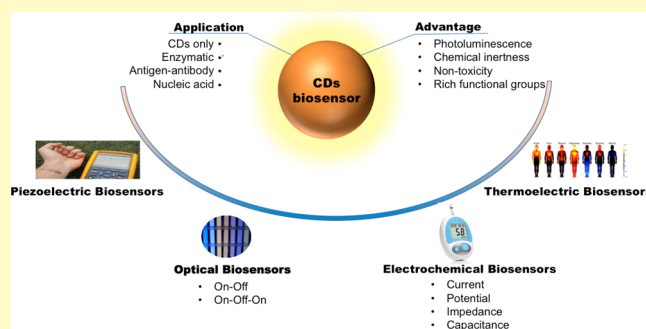
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ABSTRACT: Early diagnosis of diseases is of great importance because it increases the chance of a cure and significantly reduces treatment costs. Thus, development of rapid, sensitive, and reliable biosensing techniques is essential for the benefits of human life and health. As such, various nanomaterials have been explored to improve performance of biosensors, among which, carbon dots (CDs) have received enormous attention due to their excellent performance. In this Review, the recent advancements of CD-based biosensors have been carefully summarized. First, biosensors are classified according to their sensing strategies, and the role of CDs in these sensors is elaborated in detail. Next, several typical CD-based biosensors (including CD-only, enzymatic, antigen–antibody, and nucleic acid biosensors) and their applications are fully discussed. Last, advantages, challenges, and perspectives on the future trends of CD-based biosensors are highlighted.

KEYWORDS: carbon dots, graphene quantum dots, polymer dots, biosensor, FRET, on–off–on, fluorescence quenching, enzyme, antibody, DNA



Early diagnosis of diseases is the key to increasing the chance of successful treatments,¹ so there is an urgency to develop biosensors that can detect various biomolecules rapidly, sensitively, and reliably. Biosensors are devices used to analyze organic/inorganic molecules in living organisms. A biosensor consists of three components: a detector, which recognizes the target analyte signal; a converter, which converts the detected signal into a useful output; and a signal processor, which analyzes and processes the output signal.² The detector, as the key part of a biosensor, is usually composed of biological receptors. Based on the bioreceptors used, biosensors can be divided into several categories, such as enzymatic sensors, immunosensors, nucleic acid sensors, cell sensors, biomimetic sensors and etc.^{2–4} Since the advent of biosensors, they have developed rapidly in terms of sensitivity, selectivity, and range of applications. Biosensors have played important roles in various fields such as biomedicine,⁵ agriculture,⁶ food safety,⁷ environmental,⁸ and industrial monitoring.⁹ Nevertheless, biosensing also faces challenges, which mainly arise from the vulnerability of their bioreceptors. For example, the easy degradation of deoxyribonucleic acid (DNA) requires specific storage and analysis conditions, and poor stability of enzymes may cause ineffectiveness of sensors under high temperature.¹⁰

In recent years, the rapid development of nanotechnology has prompted biosensing into the era of nanoprobe, in which diseases can be diagnosed more effectively and promptly at the molecular and cellular level. Compared to traditional methods,

the combination of nanotechnology and biosensing has great advantages: high-throughput screening, improved detection limit, real-time analysis, label-free detection, and significantly decreased sample sizes.^{11,12} Further, nanomaterials introduced in biosensors could enhance properties of the bioreceptors, expanding their applicability significantly. As such, various nanomaterials such as metal nanoparticles,^{13–16} semiconductor-based quantum dots,^{17,18} dye-doped materials,¹⁹ carbon-based nanomaterials,^{20–22} and metal organic frameworks (MOF) and covalent organic frameworks (COF)^{23,24} have all been applied for the sensing and study of biomolecules. Nanomaterials with excellent optical and electrochemical properties are extremely successful and have been the focus of research in the field of biosensing. However, these nanomaterials-based biosensors also face some challenges. For instance, metal- and semiconductor-derived nanomaterials and quantum dots generally contain toxic heavy metals (i.e., cadmium, lead),²⁵ significantly limiting their biosensing applications due to potential hazards and high costs. Dye-doped nanomaterials are not only environmentally harmful but also subject to easy degradation, affecting their detection

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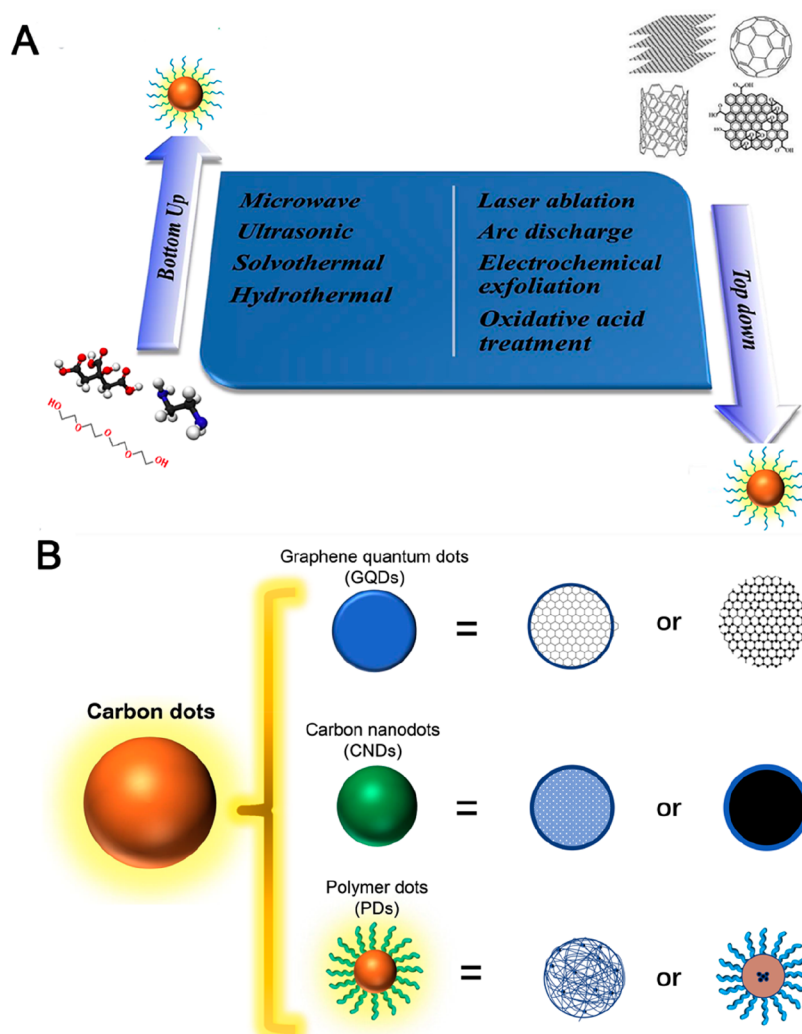


Figure 1. (A) Two approaches of CD fabrication: “top-down” (right) and “bottom-up” (left). (B) Three types of fluorescent CDs: GQDs, CNDs, and PDs, as classified by Yang and coworkers in ref 64.

accuracy.²⁶ In light of these challenges in applying traditional nanomaterials for biosensing, significant efforts have been devoted to the development of nanomaterials with better biocompatibility and photostability and less toxicity while still maintaining the excellent optical and electrochemical properties.

In this context, CDs have received enormous attention in biosensing.^{27–29} CDs are a new family member of zero-dimensional carbon-based nanomaterials with diameters less than 10 nm, known for their unique fluorescence properties. CDs were serendipitously discovered by Xu et al. when purifying single-walled carbon nanotubes (SWCNTs) by electrophoresis.³⁰ Since then, CDs have developed rapidly and become one of the research hotspots in materials science.^{31–33} Based on the nature of the carbon precursor, core structure, and quantum effect, CDs can be divided into three categories: namely, graphene quantum dots (GQDs), carbon nano dots (CNDs), and carbon quantum dots (CQDs).³⁴ GQDs are π -conjugated disc-shaped nanostructures, which are mainly generated by cutting large graphene structures. CNDs are amorphous quasi-spheres that lack quantum constraints. CQDs are spherical quantum dots with quantum confinement and crystal structure. CDs can be synthesized via a wide range of methods, and these methods

can be simply classified into two categories: namely, “top-down” and “bottom-up” (Figure 1A). The “top-down” approach refers to the decomposition/breakdown of large pieces of raw carbon materials such as carbon soot,³⁵ carbon fiber,³⁶ activated carbon,³⁷ carbon black,³⁸ graphene,³⁹ and CNTs³⁰ into CDs through a harsh process. Synthetic methods mainly include laser ablation,⁴⁰ arc discharge,³⁰ electrochemical exfoliation^{41,42} and oxidation acid treatment.^{43,44} Due to the harsh conditions applied in these reactions, high-pressure, high-temperature, and energy-consuming instruments and equipment are usually required, which lead to high costs. In most cases, the fluorescence quantum yields (QYs) of the resulting CDs are relatively low, and thus, an extra step of surface passivation is generally required. The “bottom-up” approach means the synthesis of CDs from carbon-containing molecules through a “decomposition–polymerization–carbonization” process. The carbon-containing molecules can be small organic molecules such as citric acid,^{45–47} polyols,⁴⁸ and amino acids;⁴⁹ synthetic polymers such as polyethylene glycol (PEG)^{50,51} and polythiophene;⁵² and natural products such as carbohydrates,⁵³ polysaccharides,⁵⁴ and biomass (i.e., orange juice, honey, betel nut shells, silk, etc.).^{55–59} Preparation methods mainly include pyrolysis-solvothermal,^{60,61} hydrothermal carbonization,⁶² and microwave/ultrasonication.⁶³

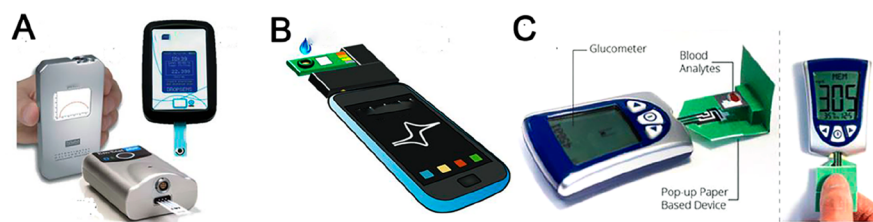


Figure 2. Portable electrochemical sensors: (A) commercially available pocket-sized potentiostats. Adapted with permission from ref 87. Copyright 2017, Wiley-VCH. (B) Smartphone-based potentiostats. Adapted with permission from ref 87. Copyright 2017, Wiley-VCH. (C) Routine blood glucose meter. Reproduced with permission from ref 90. Copyright 2016, American Chemical Society.

Due to the synthesis nature of CDs in the “bottom-up” approach, some CDs prepared from small organic molecules are regarded as fluorophores formed by cross-linking of monomers and/or linear polymers and are thus referred to as polymer dots (PDs).⁶⁴ Therefore, CDs could also be broadly classified into three types as GQD, CND, and PDs (Figure 1B).

Compared to traditional nanomaterials, CDs have various advantages in biosensing due to their unique properties. First, the unique photoluminescence (PL) property of CDs is very advantageous in biosensing. As is well-known, optical biosensors function by converting intangible information about target analytes into detectable optical signals (i.e., fluorescence intensity/wavelength, color change). In general, the wavelength of the nanoprobe should be in the near-infrared (NIR) region⁶⁵ because ultraviolet rays are harmful to living organisms,^{66,67} and visible light is easily absorbed by biological tissues. The fluorescence of CDs can be tuned from the visible to NIR range through careful carbon precursor selection,⁵² synthesis condition manipulation,^{68,69} and heteroatom doping.^{70–72} Therefore, CDs are extremely suitable nanoprobe for (optical) biosensor development. Second, chemical inertness and PL stability are also highly desired properties for nanoprobe in biosensing. Encouragingly, unlike organic dyes, CDs remain stable in the body and are not easily metabolized by cells. After sensing, they can be discharged from the body.⁷³ Further, CDs can be stored in aqueous solution for more than half a year without affecting their PL,⁷⁴ demonstrating their high PL stability with extreme long shelf life. This is very important because it can avoid most analysis errors arising from a deteriorating fluorescence probe. Third, nanoprobe applied in biosensing should be harmless to organisms. CDs, compared to traditional nanomaterials, are known for their nontoxicity due to their very nature (i.e., carbon composed).⁷⁵ Fourth, the surfaces of CDs contain a rich number of functional groups that can provide abundant binding sites for specific bioreceptors, which is essential for their application as effective nanoprobe for biosensing. Lastly, the manufacturing techniques of CDs are simple and diverse, making it highly possible to achieve mass production, which is very beneficial for the wide application and commercial development of CDs for biosensing. As such, with these unique properties and advantages, CDs demonstrate great potential as nanoprobe in biosensing.

Thanks to the above-mentioned advantages, applications of CDs in biosensing have advanced significantly in recent years. Unfortunately, reviews that focus specifically on the recent advances of CDs for biosensor development have been rather limited. Most review articles focus on the synthesis, physical and chemical properties of CDs,^{76–78} or their applications in

biomedicine and bioengineering,^{33,79–82} energy storage and conversion, and ions and molecules sensing.^{83–85} In this context, this article means to survey recent developments of CDs in biosensing: first, we will discuss various sensing strategies that have been developed in CD-based biosensing, with an emphasis on optical biosensing. In this section, two major optical biosensing strategies, namely “on–off” and “on–off–on” will be carefully discussed, and three types of fluorescence quenching mechanisms, Förster resonance energy transfer (FRET), photoinduced electron transfer (PET), and inner filtering effect (IFE) of CDs will also be introduced. Next, several typical CD-based biosensors such as enzymatic sensors, immunosensors, and nucleic acid sensors as well as other biosensors will be elaborated. The sensing methods, advantages/disadvantages, and application scopes of these biosensors will also be carefully dissected and discussed. Last, perspectives on the challenges and future trends of CD-based biosensors are also provided.

■ TYPICAL CD-BASED BIOSENSING MECHANISMS

Electrochemical Biosensing Mechanism. According to International Union of Pure and Applied Chemistry (IUPAC) recommendation, an electrochemical biosensor is a self-contained integrated device,⁸⁶ which uses bio recognition elements (i.e., bioreceptors) to provide information on target analytes, and the bio recognition elements are in direct spatial contact with the electrochemical conduction element. Electrochemical biosensors can directly convert intangible biological information into detectable electrical signals (i.e., current, potential, resistivity, capacitance, and impedance, etc.). CDs have a rich surface area and contain a large number of functional groups that can be used to modify biological receptors. At the same time, CDs are also good electronic conductors that can realize the rapid conduction of electrons between the sensing interface and the electrodes. Therefore, CDs are excellent choices for electrochemical biosensors fabrication.

An obvious and significant advantage of electrochemical biosensors is the ease of miniaturization.⁸⁷ The development of smaller and smaller biosensors is a trend for modern medical applications, the purpose of which is early diseases diagnosis and cost reduction. Thanks to the advancements in microscale and nanoscale manufacturing processes, one can easily design and fabricate electrochemical analysis tools the size of mobile phones, realizing facile testing without special training (Figure 2). The blood glucose meter invented by Clark is such an example,⁸⁸ in which the glucose detection instrument is fabricated combining an electrode modified with an enzyme and a miniature Abe sensor. Besides their well-known miniaturization ability, electrochemical biosensors are also

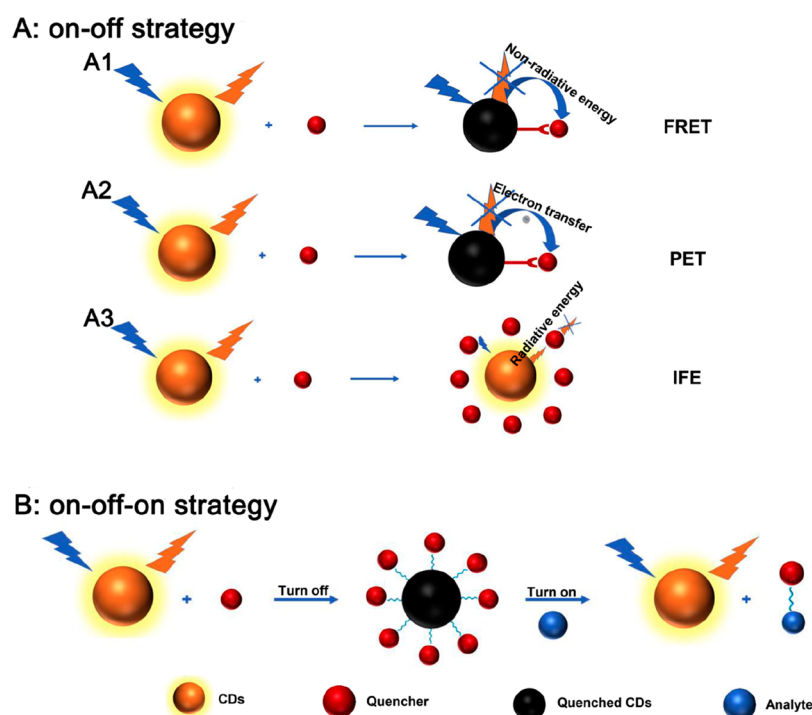


Figure 3. Typical optical biosensing strategies: (A) three fluorescence-quenching mechanisms of “on–off” strategy, (A1) FRET, (A2) PET, and (A3) IFE. (B) Scheme for “on–off–on” strategy.

famous for their strong anti-interference capability and high sensitivity. For example, a recent study has shown that the glucose electrochemical biosensor developed by Buik et al. not only effectively eliminates the interference of various substances (i.e., ascorbic acid, uric acid, acetaminophen), but also demonstrates a sensitivity more than 10 times over that of similar fluorescent sensors.⁸⁹

Optical Biosensing Mechanism. In a typical CD-based optical biosensor, the interaction of target analytes with CDs will cause the change of optical signal of CDs (i.e., fluorescence intensity variation or a shift in emission wavelength), and information about the target analytes can be provided based on the impact. Obviously, if the target analytes cannot influence the optical signal of CDs, then CDs cannot be applied as a biosensor for the analytes. Thus, in the design of a successful biosensor, the sensing mechanism/strategy should be carefully considered. In this section, the commonly used sensing strategies for CD-based optical biosensors will be closely discussed.

“On–Off” Strategy. In most cases, the interaction of target analytes with CDs would inevitably lead to the decrease of CDs’ fluorescence intensity (PL quenching); when this mechanism is utilized for biosensing, it is usually referred as “on–off” strategy/mechanism. In short, in the presence of target molecules, the PL intensity of CDs is inversely proportional to the concentration of the target analytes due to the quenching effect (Figure 3A). Based on the linear relationships, the concentration of target analytes can be readily determined using calibration curves. At present, there are mainly three mechanisms of PL quenching that have been used for the design of CD-based optical biosensors: namely FRET, PET and IFE.

In a typical FRET quenching (Figure 3A1), the nonradiative transfer of excitation energy from the excitation donor (i.e., CDs) to the proximal ground state acceptor (i.e., quencher)

occurs, causing PL quenching of CDs. There are some conditions that need to be satisfied in order for a FRET to occur, which include: 1) comparable energies for the excitation state of a donor and the ground state of an acceptor, which means the emission spectrum of the donor should overlap with the absorption spectrum of the acceptor; 2) a proper distance between the donor and the acceptor, which is normally between 1 and 10 nm; 3) The transition dipoles of the donor and acceptor molecules must be close to parallel; 4) the donor must have a sufficient fluorescence lifetime for the energy transfer to occur.⁴ As several conditions have to be met to have a FRET transfer, the choice of CDs is very important when designing a FRET quenching-based biosensor. Because the ratio of two connected fluorescence signals can be used as the detection index in a FRET system, a ratiometric fluorescence sensor can be readily designed, effectively eliminating the background interferences and fluctuations.⁹¹

In a typical PET quenching (Figure 3A2), instead of a nonradiative energy transfer between donors and acceptors, an electron transfer has occurred in which CDs serve as electron donors and target analytes as electron acceptors. Under met conditions, photon-excited electrons of CDs are transferred to the target analytes; as a result, there are less excited electrons returning from excited states to the ground state, causing PL quenching. Generally speaking, a PET quenching is mostly observed between CDs and metal ions,⁹² however, under some specific conditions, it could also occur between CDs.⁹³

Unlike FRET and PET quenching, strictly speaking, the PL of CDs is not “quenched” in an IFE process, it is simply “blocked” from the detector of a fluorometer. In the typical IFE process, the emission spectrum of donors (i.e., CDs) overlaps well with the absorptive spectrum of the acceptors (i.e., target analytes). Thus, the emitted light of CDs, before reaching the detector, is absorbed by target analytes, causing decrease of PL intensity of CDs (Figure 3A3). The degree of

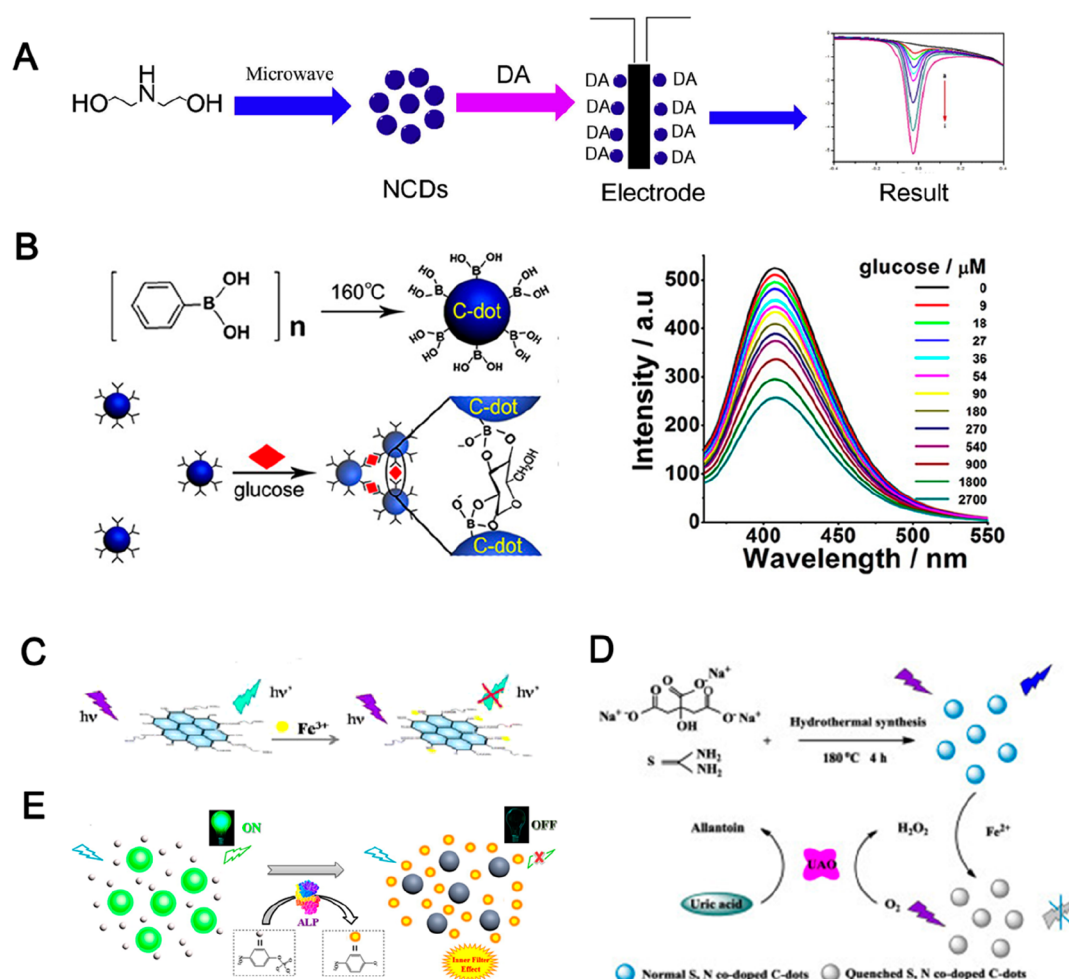


Figure 4. (A) CDs are loaded on the electrode surface for the detection of dopamine. Reproduced with permission from ref 103. Copyright 2015, Elsevier. (B) Detection of glucose by a CD-only biosensor through FRET. Reproduced with permission from ref 106. Copyright 2014, American Chemical Society. (C) Detection of Fe³⁺ by a CD-only biosensor through PET. Reproduced with permission from ref 92. Copyright 2014, American Chemical Society. (D) Uric acid detection by a CD biosensor through the Fenton reaction. Reproduced with permission from ref 111. Copyright 2016, Elsevier. (E) Detection of alkaline phosphatase by a CD biosensor through IFE. Reproduced with permission from ref 112. Copyright 2016, American Chemical Society.

fluorescence “quenching” can be then used for the detection and analysis of the target analytes.⁹⁴

“On–Off–On” Strategy. In the “on–off–on” strategy, unlike in the “on–off” strategy, the fluorescence of CDs is first turned off by nonanalyte quenchers, and then restored with the adding of the target analytes (Figure 3B). The degree of fluorescence restoration of CDs can be then used for the analysis of the target analytes. In this strategy, quenching of CDs fluorescence by nonanalyte quenchers could involve any of the above-discussed quenching mechanisms (FRET, PET or IFE). The interaction of target analyte and quencher is stronger than that of quencher with CDs, causing the release of CDs from quenchers, and thus restoring the CDs fluorescence.

As can be seen from the sensing mechanism discussed above, optical biosensors used in clinical diagnosis have many advantages that cannot be ignored. Simple operation and low production cost are among the most prominent advantages. Optical biosensors do not require complicated instruments; a benchtop fluorometer would generally meet the requirement. Sometimes, there are even optical changes that can be observed directly with naked eyes (i.e., colorimetric biosensing^{95,96}), reducing the involvement of sophisticated instru-

ments. However, when the concentration of the target analytes is low, optical biosensors normally have very poor sensitivity and are susceptible to environmental interferences.

Other Biosensing Mechanisms. Besides the above-mentioned electrochemical and optical biosensing, commonly seen biosensors also include piezoelectric biosensors, thermoelectric biosensors, etc. In a typical piezoelectric biosensor, the transducer is composed of piezoelectric material, and then the biometric material is smeared on the piezoelectric material to establish proper mechanical vibration between the two. When the target analyte interacts with the biorecognition material from a biosensor, it will cause a change in the mechanical vibration of the piezoelectric material; the mechanical vibration signal of the piezoelectric material is then converted into an electrical signal for the analysis of the target analytes. For example, Chen et al.⁹⁷ developed such a sensor from aluminum nitride and used it for the highly sensitive detection of pesticide residues. Besides the detection of important biological molecules, this biosensing method is also very useful in studying the kinetics of multiple important biological reactions such as antigen–antibody binding, protein–ligand interaction, and DNA complementation. On the other hand, thermo-

Table 1. Selected Examples of CD-Only Biosensors^a

carbon source	synthetic method	target	sensing mechanism	sensing technique	linear range ($\mu\text{mol L}^{-1}$)	LOD ($\mu\text{mol L}^{-1}$)	ref
DEA	microwave	dopamine	-	electrochemical	5.0×10^{-2} to 8.0	1.2×10^{-3}	103
carbohydrate	hydrothermal	dopamine	-	electrochemical	0 to 1×10^3	1.0×10^{-3}	104
Saccharomyces	microwave	pH; VB12	FRET	optical	-	2.19	107
DTPA	hydrothermal	curcumin	FRET	optical	$0.74\text{--}5.18 \mu\text{g mL}^{-1}$	44.8 ng mL^{-1}	113
citric acid	hydrothermal	Fe^{3+}	PET	optical	1×10^{-2} to 5×10^2	2.5×10^{-3}	92
MAC	hydrothermal	pH; Fe^{3+}	PET	optical	0–1.6	5.0×10^{-2}	102
tomato	solvothetical	Fe^{3+}	PET	optical	0.1–2	-	109
PEI	microwave	Cu^{2+}	PET	optical	1×10^{-2} to 2	6.7×10^{-4}	114
catechol	hydrothermal	ALP	IFE	optical	1×10^{-2} to 25	1.0×10^{-3}	112
citric acid	hydrothermal	hemoglobin	IFE	optical	0–6	3.4×10^{-2}	115
citric acid	hydrothermal	uric acid	Fenton	optical	8.0×10^{-2} to 50	7.0×10^{-2}	111
gelatin	hydrothermal	choline	Fenton	optical	2.5×10^{-2} to 50	2.5×10^{-2}	116
		ACh			5×10^{-2} to 50	-	
		ChOx			1–75	-	
		AChE			1–80	-	
PVP	hydrothermal	choline; ACh	Fenton	optical	0.1–40; 0.5–6	0.1; 0.5	117
PLA	hydrothermal	glucose	-	optical	9 to 9×10^2	1.5	106
tartaric acid	microwave	cysteamine	-	optical	10 to 2.1×10^2	7.54×10^{-2}	118

^aDEA: diethanolamine; DTPA: diethylenetriaminepentaacetic acid; MAC: *m*-aminobenzoic acid; PEI: poly(ethylenimine); PVP: polyvinylpyrrolidone; PLA: phenylboronic acid; VB12: cobalamin; ALP: alkaline phosphatase; ACh: acetylcholine; ChOx: choline oxidase; AChE: acetylcholinesterase. “-”: Not mentioned in the literature. LOD: Limit of detection.

electric biosensors detect target analytes by measuring temperature changes. Almost all chemical and biological reactions involve heat exchange, causing the absorption or release of heat. This promoted the birth of thermoelectric biosensors.⁹⁸ For example, Bari et al.⁹⁹ developed such a biosensor for the sensing of inflammatory cytokine tumor necrosis factor- α (TNF- α). In this sensor, an antibody is attached to the surface of the antimony/bismuth thermoelectric sensor. When the sensor is in contact with TNF- α , an antigen–antibody binding reaction occurs and heat is released due to the interaction. The thermoelectric sensor then converts the thermal signal into an electrical signal to realize the measurement of TNF- α . Thermoelectric biosensors are mainly used for the measurement of enzymatic and immune reactions.¹⁰⁰ Their main advantages are good stability and conducive to miniaturization.

■ APPLICATIONS OF CD-BASED BIOSENSORS

CD-Only Biosensors. A biosensor is mainly composed of a detector, a converter and a signal processor.² In most sensing systems, CDs function as converters, the main function of which is to convert intangible information into useful signals recognizable by the signal processor. Thanks to the rich presence of various surface functional groups, CDs are sometimes sensitive to specific biomolecules.^{101,102} This phenomenon makes it feasible to design biosensors directly with CDs without additional detectors (i.e., bioreceptors), in which CDs function as both detectors and converters simultaneously.

Electrochemical Biosensing of CD-Only Biosensors. Because of their excellent electron transport capabilities, CDs are frequently used in electrochemical biosensing. In addition, CDs could directly interact with target analytes to influence electrochemical signals of electrodes. Therefore, in an electrochemical biosensor, CDs are generally directly loaded onto an electrode surface, and the altered signals (i.e., current) due to the interaction of CDs with biomolecules are collected for the detection and analysis of the target analytes. For

example, Jing et al. designed an electrochemical biosensor for direct detection of dopamine from N-doped CDs, the synthesis of which did not involve any organic solvent or catalyst (Figure 4A).¹⁰³ The surface of the CDs contains a rich number of carboxyl groups, which interact well with dopamine through an electrostatic interaction. Thus, when an electrode loaded with CDs comes into contact with dopamine, the current of the electrode will change. And the change of current has a linear relationship with the concentration of dopamine. With this sensor, the authors were able to quickly detect the level of dopamine in human serum and urine, achieving a linear range of 5×10^{-8} to 8×10^{-6} mol/L and a detection limit of 1.2×10^{-9} mol/L. In a different study, Jiang et al. developed a similar biosensor which is capable of monitoring dopamine in real time.¹⁰⁴

Optical Biosensing of CD-Only Biosensors. CDs have unique optical properties and emit in both visible light and infrared regions,⁷⁶ thus they are frequently used in optical biosensing. The PL of CDs is mainly influenced by their sizes and surface states.^{34,64} Because the interaction of CDs with analytes could alter the surface states; the PL properties (i.e., intensity, emission wavelength, etc.) of CDs can be easily influenced.¹⁰⁵ The degree of PL alteration (i.e., fluorescence quenching) of CDs is then used to detect and analyze the target analytes in a typical CD-based optical biosensor. Depending on the nature of the analytes, there are mainly three types of fluorescence quenching (FRET, PET and IFE, see Figure 3) explored for the design of CD-only optical biosensors. For example, Shen et al.¹⁰⁶ developed a glucose biosensor from phenylboronic acid-derived CDs; due to the interaction of surface boric acid of CDs with glucose, FRET energy transfer occurs between CDs and glucose, effectively quenching the fluorescence of CDs (Figure 4B). The degree of fluorescence quenching is then used for the analysis of glucose level in body, achieving 10–250 times higher sensitivity than the previous boric acid fluorescent nanosensing detection system. Similar strategy has also been applied for the sensing of vitamin B12.¹⁰⁷ Considering the fact that CDs have very good

specificity toward some metal ions (i.e., Cu^{2+} and Fe^{3+}),^{108,109} CDs are also frequently used for the sensing of trace metal elements in organisms (Figure 4C).⁹² In most of the systems, metal ions are detected because their interaction with CDs could effectively quench the PL of CDs via PET. In a recent report, Liu and coworkers realized the sensitive detection of Fe^{3+} , however, the quenching of CDs' PL was believed to be the ferric ion-induced aggregation of CDs, not PET between Fe^{3+} and CDs.¹¹⁰

All the above-mentioned sensing systems require a quenching effect from the interaction of CDs with target analytes. However, in some occasions CDs do not interact with the target analytes, or their interaction does not lead to an observable PL quenching, rendering the current strategy ineffective. To bypass this issue, some scientists have come up with an ingenious solution: instead of targeting the analytes, they are focusing on the products of the target analytes after known reactions with clear mechanisms. With the information from the products, one can analyze the target analytes retrospectively. For example, uric acid has no observable interaction with CDs, thus there is no direct way that a CD-based biosensor could detect uric acid. However, it is known that the catalytic reaction of uric acid by uricase produces H_2O_2 , and products of the Fenton reaction (promoted by H_2O_2) could quench the PL of CDs, making the indirect detection of uric acid feasible. Indeed, Wang et al.¹¹¹ were able to develop such a biosensor and realized the sensitive detection of uric acid in human serum samples (Figure 4D). With the same strategy, Li and coworkers¹¹² realized the efficient detection of p-Nitrophenylphosphate, the analysis of which was based on the efficient IFE quenching of CDs fluorescence by the catalytic product, p-Nitrophenol (Figure 4E). In general, construction of biosensors directly from CDs could significantly reduce the complexity by avoiding tedious surface modification and bioreceptors loading, which is very advantageous in potential large-scale production and applications. Selected examples of CD-only biosensors in which external bioreceptors are not required are summarized in Table 1.

CD-Based Enzymatic Biosensors. While CDs could interact with some analytes directly and generate signal fluctuations (i.e., optical or electrochemical signals) that can be used for sensing. However, CDs do not interact with all analytes directly and direct sensing of analytes using CD-only biosensors is not always feasible. As catalytic reactions by enzymes are facile and specific, scientists have combined CDs with enzymes for the design of biosensors, in which the products (or side products) of an enzymatic reaction could interact with CDs and cause signal variation to realize an indirect detection of the target analytes.

Electrochemical Biosensing of CD-Based Enzymatic Biosensors. Enzymatic electrochemical biosensors analyze target substances by monitoring the electrochemical signals of the electrodes during an enzyme-catalyzed reaction. The introduction of CDs into these systems would significantly improve the stability and sensitivity of the biosensors, because (1) immobilization of CDs on the electrode surface provides rich binding sites for enzymes, making the absorption of enzymes on the electrode much more stable and reliable; (2) CDs are good electron transfer materials, which help the efficient transmission of electrical signals from the interfaces to the electrode. Thus, CDs have been frequently used to design enzyme-involved electrochemical biosensors. For example,

because a change in electrochemical signal is normally observed during redox reactions between oxidoreductase and its substrates, various CDs-derived biosensors have been designed to detect oxidoreductase substrates (i.e., glucose,^{89,119} H_2O_2 ,¹²⁰ and galactose¹²¹). When an electrode containing an oxidase comes into contact with a substrate-containing solution, a redox reaction occurs on the interface of the electrode and is accompanied by transfer of electrons, resulting in a change in electrode current. Thus, in these systems, the target analytes were detected and analyzed by monitoring the current. Generally speaking, biosensors based on enzymes and CDs have attracted much attention in recent years due to their high specificity and sensitivity. However, they also face some problems, for example, due to the nature of enzymes, sensors are prone to affections from temperature and pH. Also, reliable and reproducible fabrication of electrodes with known and controllable ratio of CDs and enzymes are still challenging.

Optical Biosensing of CD-Based Enzymatic Biosensors. Similar to electrochemical biosensors, most CD-based enzymatic, optical biosensors also rely on the interaction (i.e., fluorescence quenching) of CDs with the catalytic product of the target analytes for effective sensing (Figure 5A). For example, enzyme-catalyzed oxidations of substrates

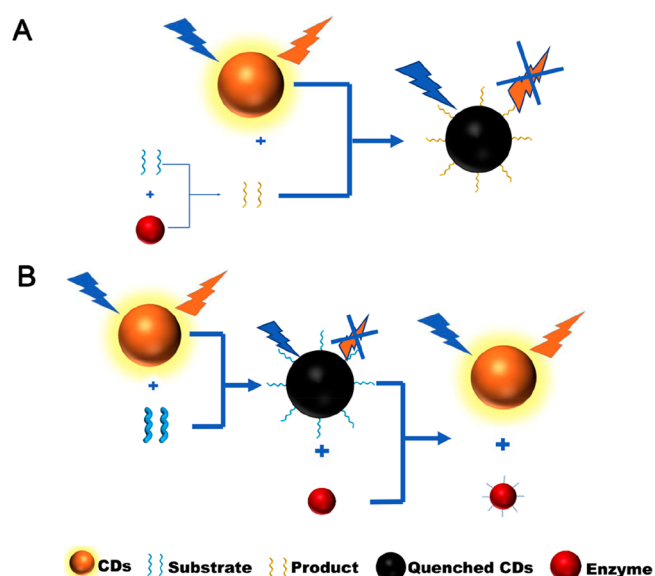


Figure 5. Two sensing strategies of CD biosensors based on enzymatic reactions.

such as acetylcholine, uric acid, and glucose could produce H_2O_2 ,^{91,111,116,122} as a result, the concentrations of these analytes can be facilely determined via monitoring the degree of fluorescence quenching of CDs arisen from the Fenton reaction of H_2O_2 (Figure 6A). In a different study, Lu et al. designed an efficient β -glucuronidase biosensor, the sensing of which was based on the IFE quenching effect of CDs by p-nitrophenol (the β -glucuronidase catalytic product of 4-nitrophenyl- β -D-glucuronide, Figure 6B).¹²³

While most biosensors of this type have been designed for the sensing of important biomolecules, recent studies have also demonstrated their unique ability in the detection and evaluation of catalytic performance of specific enzymes. For this specific purpose, the biosensor is generally designed by following the “on–off–on” strategy (Figure 5B), in which the substrate form a complex with CDs and causes the quenching

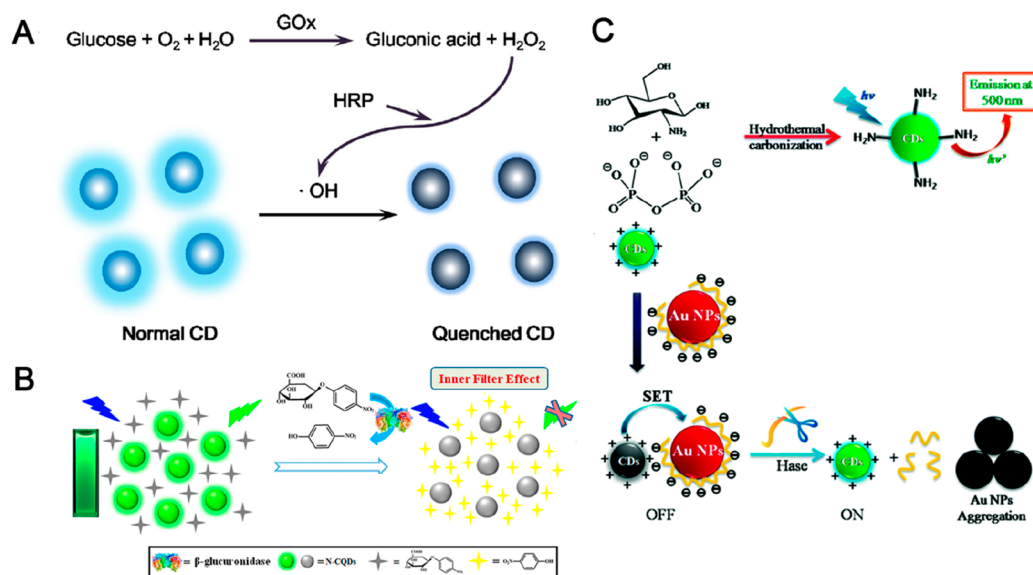


Figure 6. (A) CD biosensors are used to detect glucose and glucose oxidase. Reproduced with permission from ref 91. Copyright 2018, Elsevier. (B) CD biosensors are used to detect β -glucuronidase. Reproduced with permission from ref 123. Copyright 2016, Elsevier. (C) CD biosensors are used to detect hyaluronic acid or hyaluronidase. Reproduced with permission from ref 124. Copyright 2015, Royal Society of Chemistry.

Table 2. Selected Examples of CD-Based Enzymatic Biosensors^a

sensor platform	target	mechanism	technique	linear range	LOD	ref
GOx-CDs-AuNPs	glucose	-	electrochemical	0.16–4.32 mM	13.6 mM	89
CDs-GOx	glucose	-	electrochemical	1–12 mM	0.25 mM	119
HRP-CDs-LDHs-GC	H_2O_2	-	electrochemical	0.1–23.1 μ M	0.04 μ M	120
CDs- β -galactosidase	lactose	-	electrochemical	-	2.9×10^{-4} M	121
CDs-peptides-AuNPs	trypsin	FRET	optical	2.5–80 ng mL ⁻¹	0.84 ng mL ⁻¹	125
CDs-aptamer-GO	lysozyme	FRET	optical	0.01–2 μ g/mL	1×10^{-3} μ g/mL	126
CDs-cytochrome c	thrombin	PET	optical	0–1 μ M; 10–400 μ M	33 ng mL ⁻¹	127
CDs, Cu^{2+}	alkaline phosphatase	PET	optical	16.7–782.6 U/L	16.7 U/L	128
CDs-tyrosinase	levodopa	IFE	optical	$0.311\text{--}3.17 \times 10^2$ μ mol L ⁻¹	9×10^{-8} mol L ⁻¹	52
CDs	alkaline phosphatase	IFE	optical	0.01–25 U/L	0.001 U/L	112
CDs	β -glucuronidase	IFE	optical	1–60 U L ⁻¹	0.3 U L ⁻¹	123
CDs-HA-AuNPs	hyaluronidase	SET	optical	0–100 U mL ⁻¹	1 U mL ⁻¹	124
CDs, uricase	uric acid	Fenton	optical	0.08–50 mM	0.07 mM	111
	choline			0.025–50 μ M	0.025 μ M	
	acetylcholine			0.05–50 μ M	-	
CDs	choline oxidase	Fenton	optical	1–75 U/L	-	116
	acetylcholinesterase			1–80 U/L	-	
CDs, rhodamine 6G	glucose	-	optical	0.1–500 μ M	0.04 μ M	91
GQDs-GOx-HRP-PO	glucose	-	optical	0.2–10 μ mol/L	0.08 μ mol/L	122
CDs-ssDNA- Tb^{3+}	glucose	-	optical	1–10 μ M	0.06 μ M	129
TiO ₂ -CDs-GOx	glucose	-	PEC	0.1–18 mM	0.027 mM	130
WS ₂ -CDs-AuNPs-DNA	T4- β -glucosyltransferase	-	PEC	0.1–220 units/mL	0.028 unit/mL	131
	5-hydroxymethylcytosine	-		0.01–100 nM	0.0034 nM	

^aGOx: glucose oxidase; AuNPs: gold nanoparticles; HRP: horseradish peroxidase; LDHs: layered double hydroxide composites; GC: glass carbon electrode; HA: hyaluronate; ssDNA: single-stranded DNA; PO: phenol; WS₂: tungsten disulfide; SET: surface energy transfer; PEC: photoelectrochemical.

of CDs fluorescence. In the presence of the desired enzyme, the substrate is decomposed, restoring the fluorescence of CDs. Thus, based on the degree of CDs fluorescence recovering, the presence and concentration of target enzyme can be determined. For instance, a trypsin biosensor was constructed according to such strategy. In this system, CDs and AuNPs were brought to proximity by peptide chains, resulting in the quench of CDs PL; in the presence of trypsin, peptide chains are destroyed to distance CDs and AuNPs,

effectively restoring the PL of CDs.¹²⁵ In a similar study, a hyaluronidase biosensor was constructed from positively charged CDs and hyaluronic acid coated AuNPs (negatively charged), in which the fluorescence of CDs was quenched by AuNPs through surface energy transfer (SET). However, with the introduction of hyaluronidase, the fluorescence of CDs was effectively recovered due to the decomposition of hyaluronic acid (Figure 6C).¹²⁴ In general, due to the specificity of enzyme-catalyzed reactions, CD-based enzymatic biosensors

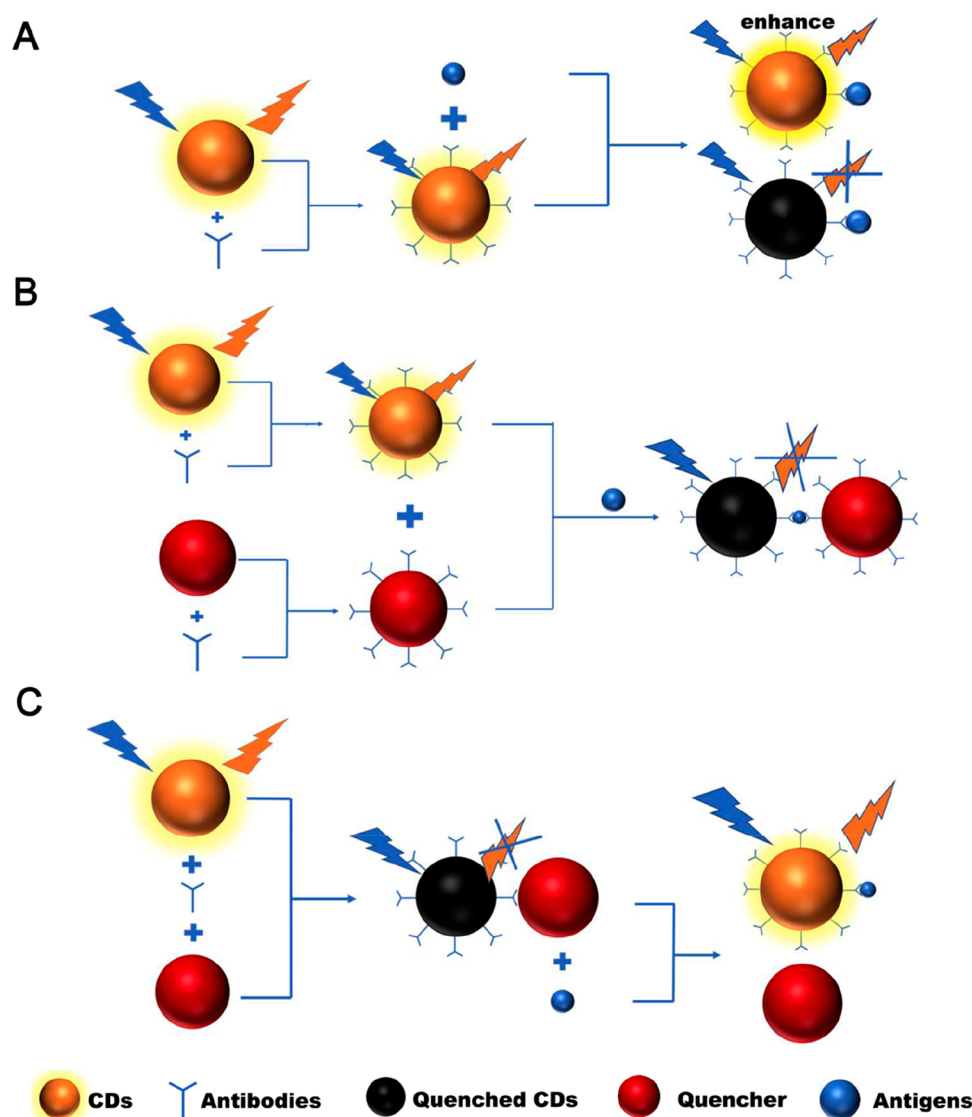


Figure 7. Three sensing strategies of CD biosensors based on antigen–antibody interaction.

tend to have very high sensing specificity, rendering them very useful biosensors for specific biomolecules and enzymes detection. Selected examples of CD-based enzymatic biosensors are summarized in Table 2.

CD-Based Antigen–Antibody Biosensors. Most substances can interact directly with CDs or generate usable signals in presence of enzymes; however, species such as viruses, bacteria, and some specific proteins neither interact directly with CDs nor are sensitive to enzymes. For this type of sensing applications, the highly specific binding of antigen–antibody turned out to be the best choice for biosensor design. Sensors designed based on the interaction of antigen–antibody are commonly referred as immunosensors. Antibodies were first applied for biosensing back in 1950, paving the way for the rapid development of immunodiagnosis.¹³² Since then, scientists around the world have spent enormous efforts for the development of various antigen–antibody biosensors for clinical diagnosis. Antigens are a class of substances (i.e., viruses, bacteria, proteins) that can stimulate immune systems and produce antibodies. According to the chemical structure and biological function of antigens, they can be divided into five broad classes: IgG, IgM, IgA, IgE, and IgD.¹³³ Antibodies

are composed of polypeptide chains interconnected by disulfide bonds. An antibody normally contains two domains, namely variable domain and fixed domain,¹³⁴ in which the variable domain is responsible for the high selectivity toward antigens. Thanks to the rich and tunable surface functional groups of CDs to provide many active sites, it is very facile to load antibodies onto CDs in a controllable manner, either via noncovalent interactions or covalent bonding.

Electrochemical Biosensing of CD-Based Antigen–Antibody Biosensors. Electrochemical and optical biosensors are among the most commonly applied ones for CD-based antigen–antibody biosensing. An electrochemical biosensor realizes the sensing of antigens with high sensitivity and specificity through the changes of electrical signals generated upon the biorecognition between the antigen and antibody. In a CD-based antigen–antibody biosensor, one important issue to consider is that after CDs and antibody are loaded on the electrode surface, the bioactivity of the antibody needs to be retained. Such biosensors have been successfully applied for the detection of alpha-fetoprotein,¹³⁵ carcinoembryonic antigen¹³⁶ and vitamins.¹³⁷ In these systems, CDs not only accelerated the electron transfer between the sensing interface

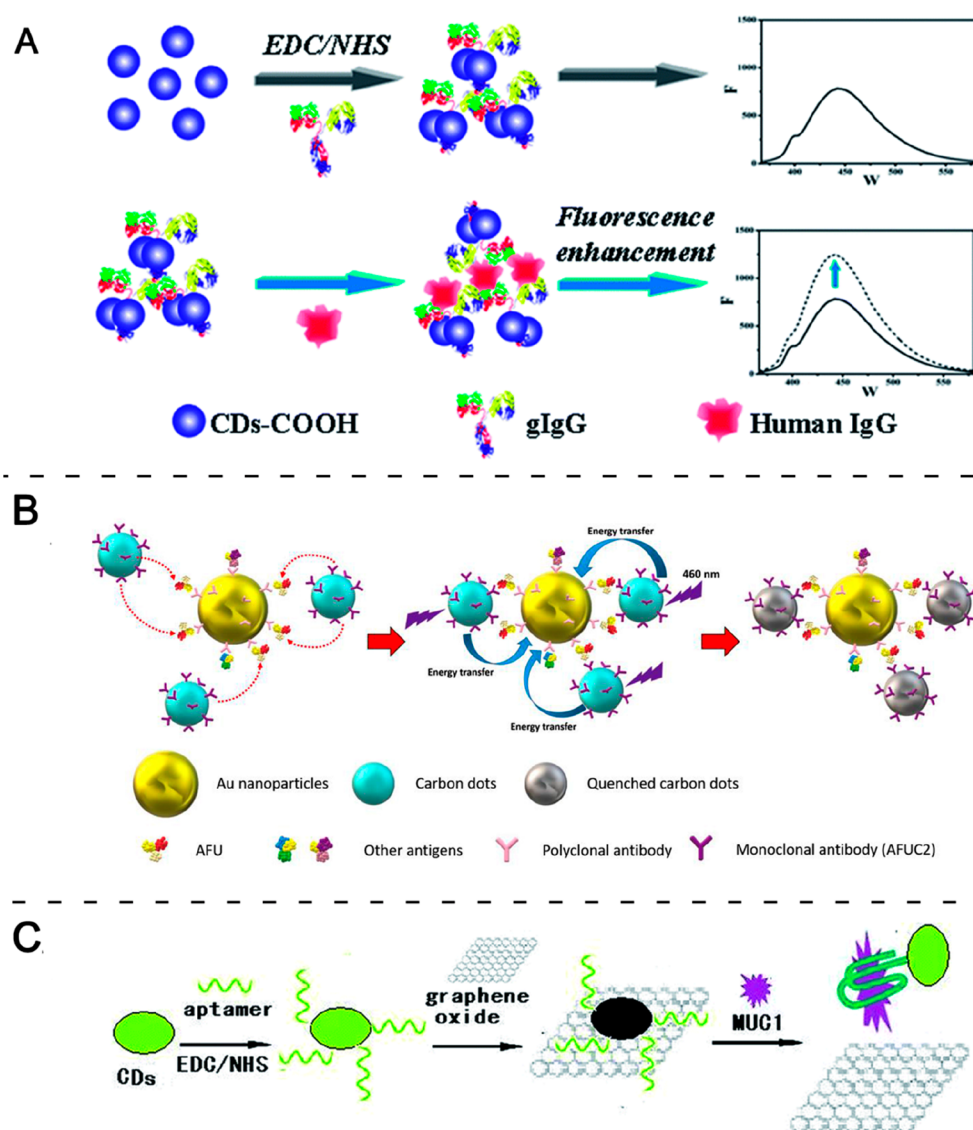


Figure 8. (A) Antigen–antibody-based CD biosensor with the addition of antigen to enhance the fluorescence of CDs. Reproduced with permission from ref 138. Copyright 2014, Royal Society of Chemistry. (B) “On–off” type antigen–antibody CD biosensor for detection of alpha-L-fucosidase. Reproduced with permission from ref 140. Copyright 2018, Elsevier. (C) “On–off–on” type antigen–antibody CD biosensor for the detection of Mucin 1. Reproduced with permission from ref 144. Copyright 2015, Royal Society of Chemistry.

and the electrode, but also increased the surface area of the electrode, resulting in much enhanced sensing efficiency. Due to the high specificity and sensitivity of antigen–antibody interaction, some of the biosensors demonstrate great potential in real clinical sample analysis. For example, the sensor developed by Gao et al. was successfully applied for alpha-fetoprotein detection in clinical blood samples, achieving a linear range of 100 fg mL^{-1} to 100 ng mL^{-1} , and a detection limit of 0.025 pg mL^{-1} .¹³⁵

Optical Biosensing of CD-Based Antigen–Antibody Biosensors. Optical biosensors are widely used because of their simple operation and ease of fabrication. CD-based antigen–antibody optical biosensors are generally composed of CDs and a specific antibody conjugated together; in the presence of a target antigen, the strong antigen–antibody interaction would have an effect (enhancement or quenching) on the fluorescence behavior of CDs. By monitoring CDs fluorescence, a highly specific and sensitive detection of antigen would be realized (Figure 7A). Biosensor developed by

Zhu et al. for the detection of human immunoglobulin (IgG) is such an example (Figure 8A), in which the fluorescence intensity of CDs was enhanced due to antigen–antibody interaction.¹³⁸ However, not all CDs emissions are directly sensitive to antigen–antibody interactions; to generate observable signals, external CDs PL quenchers (i.e., AuNPs) are often introduced. In presence of the target antigen, a CDs–antibody–antigen–antibody–quencher sandwich structure is formed, which brings CDs and quencher to close proximity for an effective PL quenching (Figure 7B). By monitoring the degree of CDs PL quenching, the presence and concentration of target antigen could be determined.¹³⁹ For instance, Leblanc and coworkers designed such a biosensor for the sensitive detection of alpha-L-fucosidase in human blood (Figure 8B). Because alpha-L-fucosidase is a key biomarker for early stage liver cancer, this sensor is promising for early diagnosis of hepatocellular carcinoma.¹⁴⁰ Many cancers have unique biomarkers, thus accurate detection of these biomarkers would be a highly potential solution for early cancer diagnosis.

Table 3. Selected Examples of CD-Based Antigen–Antibody Biosensors

sensor platform ^a	target	mechanism	technique	linear range	LOD	ref
PAMAM-CDs/AuNPs	alpha-fetoprotein	-	electrochemical	100 fg mL ⁻¹ to 100 ng mL ⁻¹	0.025 pg mL ⁻¹	135
BSA-Ab-VD ₂ -CD-CH-ITO	vitamin D2	-	electrochemical	10–50 ng mL ⁻¹	1.35 ng mL ⁻¹	137
CDs-Ab, AuNPs-Ab	alpha-L-fucosidase	FRET	optical	11.3–200nM	3.4 nM	140
CDs-Ab-CA 15-3	CA 15-3	FRET	optical	1.1 μM mL ⁻¹ to 5.0 mU mL ⁻¹	0–9 mU mL ⁻¹	141
CD-aptamer	CA125	FRET	optical	1.0 fg/mL to 1.0 ng/mL	0.5 fg/mL	142
CDs-aptamer-GO	Mucin 1	FRET	optical	20.0–804.0nM	17.1 nM	144
Ab-CDs-MoS ₂ -ssDNA	CA 125	FRET	optical	0.05–8pg/mL	5.0 fg/mL	145
	CA 15-3			1.0–35pg/mL	0.5 pg/mL	145
CDs-Ab	immunoglobulin G	-	optical	0.05–2.0 mg·mL ⁻¹	0.01 mg·mL ⁻¹	138
CQDs/AuNPs	CA 19-9	-	optical	0.01–350 U mL ⁻¹	0.007 U mL ⁻¹	139
CDs-DNA, CDs-Ab	alpha fetal protein	-	optical	0.0005–5 ng/mL	94.3 fg/mL	143
AuNPs-Ab-DNA, CDs	α-fetoprotein	-	optical	1.0 fg/mL to 1.0 ng/mL	0.2 fg/mL	146
Ab1-CD-SNPs, Ab2- FITC	α-fetoprotein	-	optical	0.317–35 μg dL ⁻¹	0.3175 μg dL ⁻¹	147
CDs-Ab, CDs- aptamer	Mucin 1	-	optical	5–100 nM	2 nM	148
CDs/g-C ₃ N ₄	PSA	-	photoelectrochemical	0.02–100 ng mL ⁻¹	5.0 pg mL ⁻¹	149

^aPAMAM: polyamidoamine dendrimers capped; BSA: bovine serum albumin; Ab: antibody; VD₂: vitamin D₂; CH: chitosan; ITO: indium tin oxide; CA 15-3, CA 125, CA 19-9: tumor markers; GO: graphene oxide; FITC: fluorescein isothiocyanate; SNPs: silica nanoparticles; g-C₃N₄: graphitic carbon nitride; PSA: prostate-specific antigen.

Encouragingly, the detection of breast cancer cell marker CA 15-3,¹⁴¹ ovarian cancer cell marker CA-125,¹⁴² and liver cancer cell marker alpha-fetoprotein¹⁴³ has all been achieved using similar methods.

The “on–off–on” strategy is also commonly explored in CD-based antigen–antibody biosensors (Figure 7C). For example, Ding and coworkers reported such a biosensor for the detection of Mucin 1.¹⁴⁴ In this biosensor, the PL of antibody-modified CDs was effectively quenched by GO due to FRET energy transfer. However, because the binding force of Mucin 1 with the antibody is much stronger than that of GO with the antibody, in the presence of the antigen–Mucin 1, CDs and GO were distanced enough to eliminate the FRET quenching effect (Figure 8C). By monitoring the PL recovery of CDs, the presence and concentrations of Mucin 1 could be facilely determined. Because Mucin 1 is overexpressed in cancer cells, thus this sensing method can also be applied for early diagnosis of cancers. Due to the very nature of antigen–antibody interaction, CD-based antigen–antibody biosensors generally have very high sensitivity and specificity toward the target analytes. Furthermore, many cancer cells have unique biomarkers, and antigen–antibody biosensors have been widely used to detect these biomarkers, paving the way for efficient and reliable early cancer diagnosis. Selected examples of CD-based antigen–antibody biosensors are summarized in Table 3.

CD-Based Nucleic Acid Biosensors. For the purpose of specific gene sequence detection, neither enzymatic reactions nor antigen–antibody interactions can be utilized. The only feasible solution is the principle of DNA complementation because the unique nucleotide sequence of DNA is highly specific. Considering the superior properties of CDs and the fact that CDs show very high affinity toward DNA through either π – π stacking or electrostatic interaction, the combination of CDs and probe DNA for biosensor development has received extensive attention. For instance, by coating CDs and probe DNA onto an Au electrode, a sensitive DNA biosensor using the principle of DNA complementation was constructed.¹⁵⁰ In this sensor, the probe DNA serves as a detector, and CDs are responsible for the electronic conduction between the sensing interface and the electrode. Because the complex-

ation of the probe DNA strand with the target DNA strand would cause an electrochemical signal change, the presence and concentration of the target DNA strand can be determined by simply monitoring the electrochemical signals (Figure 10A).

Like in other types of biosensors, the “on–off–on” strategy is also often used in nucleic acid biosensors. Due to the unique matching structures of DNA strands, the binding force of two single-stranded DNAs for DNA hybridization is much stronger than that of a single-stranded DNA with a quencher. Thus, in the presence of the target DNA strand, the already-quenched CDs PL is easily recovered because of the falling off of the quencher due to the strong complexation tendency of the DNA strands (Figure 9). Using this strategy, Mohammadi et al.

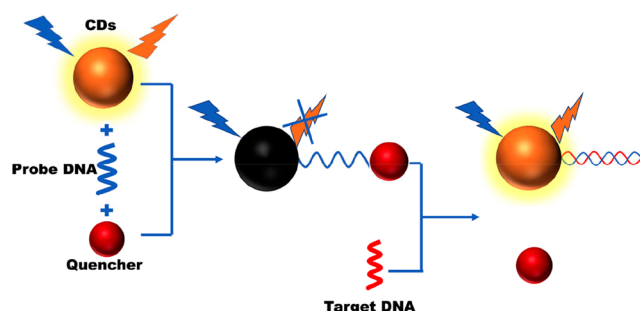


Figure 9. Scheme showing the sensing strategy of a CD-based biosensor relying on DNA strand interaction

designed an effective biosensor for the detection of cancer biomarker microRNA.¹⁵¹ In this sensor, a FRET energy transfer with MnO₂ nanosheets first quenched the PL of CDs, and the detection of microRNA was realized by monitoring the degree of CDs fluorescence recovery (Figure 10B). DNA transcribes RNA and RNA translates into proteins, thus some oligonucleotide or peptide molecules (aptamers) also bind well with DNA strand. The different interaction ability of DNA single strand and probe aptamer with carcinoembryonic antigen (CEA) was delicately exploited by Shao and coworkers for the efficient detection of CEA in pleural effusion.¹⁵² When the CDs-DNA biosensor was exposed to CEA, the fluorescence of CDs quenched by Au nanorods was restored (Figure 10C),

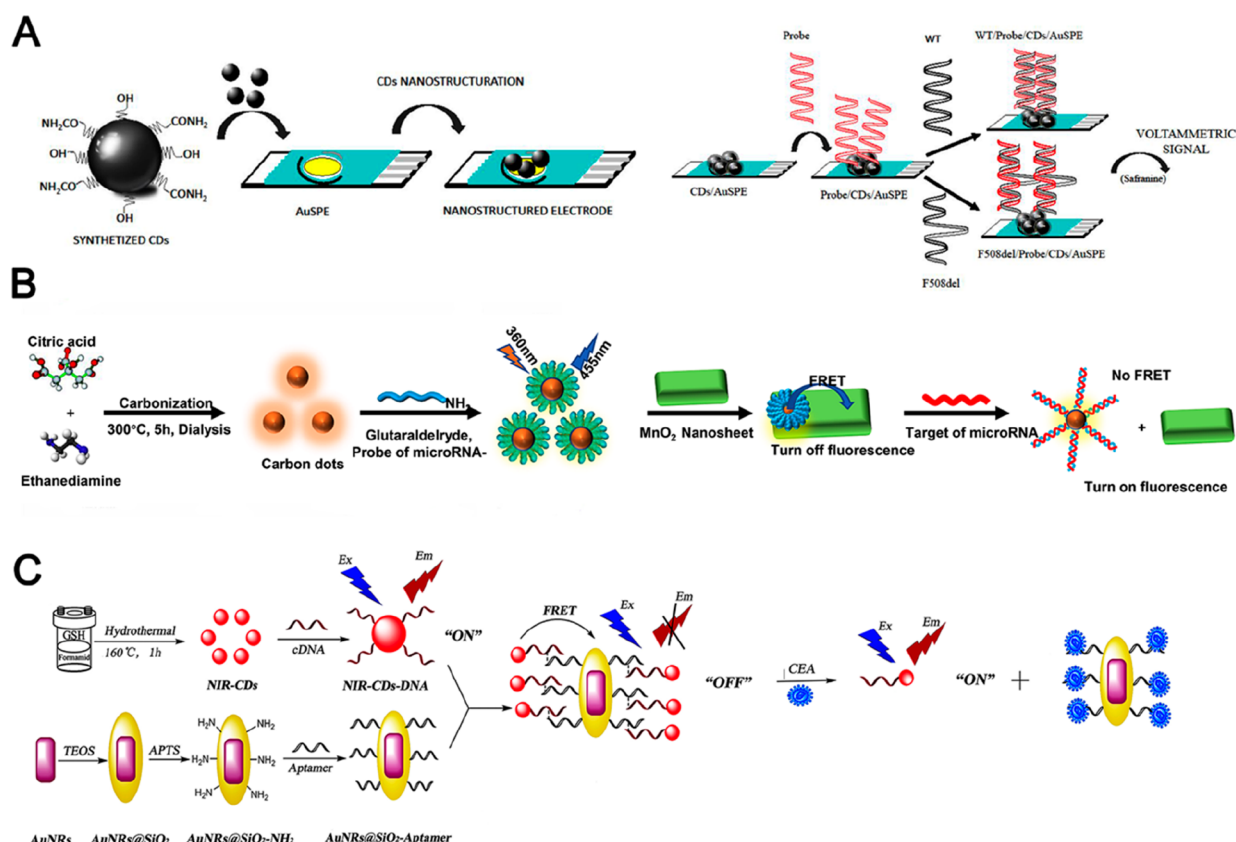


Figure 10. (A) CD-based electrochemical biosensor for DNA detection. Adapted with permission from ref 150. Copyright 2017, Elsevier. (B) CD-based optical biosensor for the detection of cancer biomarker microRNA as described in ref 151. (C) CD-based optical biosensor for the detection of carcinoembryonic antigen. Adapted with permission from ref 152. Copyright 2019, Elsevier.

Table 4. Selected Examples of CD-Based Nucleic Acid Biosensors^a

sensor platform	target	mechanism	technique	linear range	LOD	ref
AuSPEs-CDs-ssDNA	F508del	-	electrochemical	-	0.16 nM	150
CDs-AgNPs	distinguish DNA	-	electrochemical	10 ⁻¹⁶ to 10 ⁻¹¹ M	-	155
CDs-ssDNA-MnO ₂	microRNA-155	FRET	optical	0.15–1.65 aM, 1.65–20 aM	0.1 aM	151
aptamer-CDs-ssDNA	acetaminophen	FRET	optical	0.2–20 ng/L	0.04 ng/L	156
CDs-AuNPs-ssDNA	BRCA1-RNA/DNA	FRET	optical	4–120 nM	1.5 nM/2.1 nM	157
CDs-ssDNA-AuNPs	TK1-RNA/DNA	FRET	optical	10–120 nM	3.6 nM/4.5 nM	158
ssDNA-CDs-DSA	HIV	FRET	optical	50.0 fM to 1.0 nM	15 fM	159
ssDNA-CDs-DSA	exosomal microRNA	FRET	optical	-	3.0 fM	159
CDs-MB-BHQ1	microRNA-21	FRET	optical	5–160 nM	0.3 nM	160
CDs, CdTe	HIV	PET	optical	0–50 nM	1.0 nM	161
GO-CDs-Apt-CQDs-ssDNA	adenosine	-	optical	5.0 × 10 ⁻¹³ to 5.0 × 10 ⁻⁹ mol/L	2.1 × 10 ⁻¹³ mol/L	162
C-dots-NPG-ssDNA	DNA	-	optical	10 ⁻¹⁴ to 10 ⁻¹⁸ M	8.56 × 10 ⁻¹⁹ M	163
CDs-ssDNA	histone	-	optical	-	0.2 ng mL ⁻¹	164
CDs-ssDNA	carcinoembryonic antigen	-	optical	1–0.5 ng mL ⁻¹	0.3 ng mL ⁻¹	165

^aAuSPEs: integrated screen-printed gold electrodes; AgNPs: silver nanoparticle; DSA: 5,7-dinitro-2-sulfo-acridone; MB: molecular beacon; BHQ1: Black Hole Quencher 1; Apt: adenosine polymers; NPG: nanoporous gold; F508del: mutation in the CFTR gene; BRCA1: breast cancer; TK1: thymidine kinase.

and the recovery of fluorescence intensity is proportional to the concentration of CEA.

CD-based nucleic acid biosensors have various advantages such as high sensing specificity, ability of real-time analysis, and ease of miniaturization.¹⁵³ However, DNA is prone to degradation, thus specific storage and analysis conditions are generally required. Furthermore, the sensing effectiveness of these biosensors is highly influenced by sensing environment (i.e., temperature and pH).¹⁵⁴ Therefore, it is necessary to

devote extra efforts in controlling temperature and adjusting pH for maximum performance. Selected examples of CD-based nucleic acid biosensors are summarized in Table 4.

CONCLUSION AND PERSPECTIVES

Herein, the rapid advancements of CDs for biosensing in recent years have been carefully reviewed. We first briefly discussed the development of biosensing in the nano-technology age. Then, we introduced various sensing

mechanisms for different types of CD-based biosensors (i.e., electrochemical, optical, piezoelectric, and thermoelectric biosensors). Among them, the sensing strategies of CD-based optical biosensors have been closely looked at, which mainly include “on–off” and “on–off–on” strategies. In both strategies, quenching of the fluorescence of CDs is a key step, which can be realized through mainly three mechanisms, namely FRET, PET, and IFE, depending on the nature of CDs and the target analytes. Last, several types of CD-based biosensors and their applications were carefully discussed, which include (1) CD-only biosensors; (2) CD-based enzymatic biosensors; (3) CD-based antigen–antibody biosensors, and (4) CD-based nucleic acid biosensors. Due to their superior PL properties, excellent chemical stability, outstanding biological compatibility, rich and tunable surface functionalities, and their easy and economic accessibility, CDs have demonstrated enormous advantages in biosensing and related fields. First, with excellent intrinsic fluorescence, there is no need to load external fluorescent groups to CDs when fabricating the biosensors, significantly reducing the complexity of the system and difficulty of operation. Second, with strong resistance to photobleaching, it is very beneficial for the reduction of detection errors and long-term storage. Third, with rich, tunable surface functional groups, it is facile for CDs to load with various receptors and functionalities (i.e., enzymes, antibodies, and nucleic acids). Lastly, the nontoxic nature and excellent biocompatibility of CDs is beneficial for their biological applications in the real world.

Furthermore, considering some CDs possess very unique properties, if these CDs were used for biosensor development, some interesting and important biosensing might be feasible. For example, some CDs have been reported to possess intrinsic penetration ability to cross the blood–brain barrier;^{31,166,167} thus, it would be very interesting and exciting to use these CDs to develop biosensors that could eventually realize the *in vivo* sensing of brain-related diseases. In addition, some CDs have very high affinity and specificity toward specific tissues (i.e., bones);^{168–170} thus, it is also possible to develop biosensors that have very high tissue specificity, significantly expanding the practical scope and sensing targets of current CD-based biosensing. Last, CDs are reported to have good interactions with biological cells¹⁷¹ and organelles;¹⁷² thus, biosensors exploiting these unique interactions would also be very interesting and potentially greatly expand the current sensing scope. Currently, most sensing techniques realize the detection of target analytes by combining CDs with enzymes, antibodies, and nucleic acids. It would be very interesting to develop CDs with some intrinsic properties that could mimic those of biomacromolecules (i.e., enzymes, antibodies, and nucleic acids), thus realizing most detections in a label-free fashion.

Despite the above-mentioned advantages, there are still many more efforts that need to be devoted to realize the full potential of CD-based biosensors. First, the continuous emergence of vastly different carbon precursors and synthetic methods for the preparation of CDs has made the attempt to study the structure, property, and application of CDs in a standardized context extremely difficult. Sometimes, CDs from different research groups may demonstrate different properties even though they are prepared with the same carbon precursors and synthetic methods.^{47,173} Thus, when CDs are applied as biosensors, their detection capabilities and effects are quite different, significantly limiting their commercial applications. To further explore the full potential of CD-based

biosensors, the synthesis of CDs with stable, reproducible, and standardizable properties should be emphasized and pursued. Second, the bioenvironment is a very complex system, which not only contains the target analytes but also includes a lot of similar substances. Thus, strong interferences are generally expected for biosensing in real samples. As a key feature, CDs are known for the rich presence of various functional groups on their surface, which is essential for facile CD surface modification and functionalization (i.e., loading of bioreceptors). However, this feature also causes some challenges because the various functions on the CDs surface might interact with some of the substances in the bioenvironment, resulting in false signal or intense interference. Thus, while trying to maintain the presence of enough surface groups as handles for CD modification and functionalization, CDs with strong anti-interference features and capability should be targeted. Lastly, even though CD-based optical biosensors have advanced rapidly, the development of other types of biosensors (i.e., electrochemical biosensor) has been relatively slow. This situation needs to be improved because optical biosensors and other type of biosensors are complementary. For example, optical biosensors generally have low detection limits and are prone to interference from the external environment, while other types of biosensors (i.e., electrochemical biosensor) are very sensitive and normally have excellent detection limits and strong anti-interference capability. In addition, their miniaturization and corresponding miniaturization techniques are also relatively mature. As such, many more efforts should be devoted to the development of other types of CD-based biosensors (i.e., electrochemical biosensors), not only optical biosensors.

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Notes

The authors declare no competing financial interest.

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■ VOCABULARY

Nanomaterial, materials that have at least one dimension in the nanometer size (1–100 nm) in a three-dimensional space or are composed of them as basic units; Biological receptors, any biological macromolecules that can bind to hormones, neurotransmitters, drugs or intracellular signaling molecules and can cause changes in cell function; Nanoprobe, a detector made of nanomaterials for detecting microscopic substances; High-throughput screening, the method of detecting tens of millions of samples at the same time based on experimental methods at the molecular level and at the cellular level, and obtaining the corresponding data; Photoluminescence, the phenomenon that molecules or materials could emit specific light upon absorption of incoming lights, generally include fluorescence and phosphorescence

■ ABBREVIATIONS

CDs, carbon dots; MOF, metal organic frameworks; COF, covalent organic frameworks; SWCNTs, single-walled carbon nanotubes; GQD, graphene quantum dots; CND, carbon nanodots; CQDs, carbon quantum dots; QYs, fluorescence quantum yields; PEG, polyethylene glycol; PDs, polymer dots; NIR, near-infrared; FRET, Förster resonance energy transfer; PET, photoinduced electron transfer; IFE, inner filtering effect; IUPAC, International Union of Pure and Applied Chemistry; TNF- α , tumor necrosis factor- α ; SET, surface energy transfer; CEA, carcinoembryonic antigen

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